# QUANTIFICATION OF BIOACTIVE COMPOUNDS FROM *AVERRHOA BILIMBI*

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## ABSTRACT

*Averrhoa bilimbi* has been widely used in traditional medicine, thus, this fruits have received much attention because of its nutritional and antioxidant properties. The purposes of this study are to determine the bioactive compounds in *averrhoa bilimbi* and to study their optimum parameters during extraction. Antioxidant activity of the *averrhoa bilimbi* extract was determined based on 2,2-diphenyl-1-picrylhydrazyl freeradical (DPPH·), total phenolic content was measured using Folin–Ciocalteu reagent, while flavonoids is determined spectrophotometrically. Solvent extraction selected is methanol (50%) where this condition has yielded total flavonoids content is about 568.75 μg/ml QE. The highest antioxidant capacities measured is 80.02 % and total phenolic content shows about 175.3 mg/mL GAE.In this study, 60 minutes extraction time generally showed the highest effect on total flavonoids which is 496.25 μg/ml QE. *Averrhoa bilimbi* extraction had total phenolic about 164.7 mg/mL GAE and antioxidant activity is around 74.5%. 70° C extraction temperature shown the best extraction for total phenolics at 127.7 mg/ml GAE, while achieved highest total flavonoids at 656.25 μg/ml QE and the highest for DPPH radical scavenging activity is found about 70.05%. From the effect of agitation speed, 300 rpm achieved the highest value for total phenolics, total flavonoids and antioxidant capacity, DPPH which is about 193.3 mg/ml GAE, 717.75 μg/ml QE, and 77.03% respectively. Overall, based on the ideal extraction conditions chosen, optimum level of TPC, TFC and antioxidant capacity were obtained in *averrhoa bilimbi* fruit extract. The selected extraction conditions could be used for further studies and functional food product development

**KUANTIFIKASI SEBATIAN BIOAKTIF DARIPADA *AVERRHOA BILIMBI***

## ABSTRAK

*Averrhoa bilimbi* telah digunakan dengan secara meluas dalam perubatan tradisional dan secara tidak langsung, buah ini mendapat perhatian disebabkan kandungan nutrisi dan ciri-ciri antioksidannya. Objektif kajian ini adalah untuk menentukan sebatian bioaktif dalam *averrhoa bilimbi* dan mengkaji parameter optimum semasa pengekstrakan. Aktiviti antioksidan dalam *averrhoa bilimbi* ditentukan dengan 2,2-difenil-1-picrylhydrazyl radikal bebas (DPPH·), jumlah kandungan fenolik diukur menggunakan reagen manakala flavanoid ditentukan dengan. Pelarut untuk pengekstrakan yang dipilih ialah methanol (50%) dimana keadaan ini telah menghasilkan jumlah kandungan flavonoids sebanyak 568.75 μg/ml QE. Kapasiti antioksidan yang tertinggi dicatatkan pada 80.02% dan jumlah kandungan fenolik ialah sebanyak 175.3 mg/mL GAE. Dalam kajian ini, 60 minit masa pengekstrakan mencatatkan jumlah flavonoid yang paling tinggi iaitu 496.25 μg/ml QE. Pengekstrakan *averrhoa bilimbi* mempunyai jumlah fenolik sebanyak 164.7 mg/mL GAE dan aktiviti antioksidan adalah diantara 74.5%. 70° C suhu pengekstrakan menunjukkan pengekstrakan terbaik untuk jumlah fenolik iaitu 127.7 mg/ml GAE, mencapai jumlah flavonoid tertinggi pada 656.25 μg/ml QE dan yang paling tinggi untuk DPPH dicatatkan sebanyak 70.05%. Daripada kesan kelajuan pergolakan, 300 rpm mencapai nilai tertinggi bagi jumlah fenolik jumlah flavonoid dan kapasiti antioksidan masing-masing sebanyak 193.3 mg/ml GAE, 717.75 μg/ml QE, dan 77.03%. Secara keseluruhan, berdasarkan keadaan pengekstrakan ideal yang dipilih, tahap optimum jumlah kandungan fenolik, jumlah kandungan flavonoids dan kapasiti antioksidan diperoleh dalam pengekstrakan buah *averrhoa bilimbi*. Keadaan pengekstrakan yang dipilih boleh digunakan untuk kajian lanjut dan pembangunan produk makanan.

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| AC | - | Antioxidant Capacity |
| $AlCl_3$ | - | Aluminium Chloride |
| CE | - | Catechin equivalent |
| DPPH | - | 2,2-diphenyl-1-picrylhydrazyl freeradical scavenging assay |
| FC | - | FolinCiocalteu's |
| GAE | - | Gallic Acid equivalent |
| MeOH | - | Methanol |
| $NaCO_3$ | - | Sodium Carbonate |
| $NaNO_2$ | - | Sodium Nitrate |
| NaOH | - | Sodium Hydroxide |
| QE | - | Quercetin equivalent |
| TFC | - | Total Flavonoids Content |
| TPC | - | Total Phenolics Content |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| % | - | percentage |
| °C | - | degree Celsius |
| atm | - | atmospheric pressure |
| $C_A$ | - | molar concentration of solute *A* in the solution (kg mol *A*/$m^3$) |
| $C_{AS}$ | - | saturation solubility of the solute *A* (kg mol/$m^3$) |
| cm | - | centimeter |
| $D_{BA}$ | - | diffusivity of solute in the solvent ($m^2$/s) |
| Ft | - | feet |
| g | - | Gram |
| In | - | Inches |
| $k_L$ | - | mass transfer coefficient (m/s) |
| L | - | Litre |
| m | - | Metre |
| M | - | Molarity (mol/$m^3$) |
| mg | - | miligram |
| mL | - | mililiter |
| mm | - | milimeter |
| $N_A$ | - | rate of dissolution of the solute *A* in the solution (kg mol/s) |
| nm | - | nanometer |
| rpm | - | revolutions per minute (r/min) |
| *t* | - | Time (h or min) |
| w/v | - | weight / volume (kg/$m^3$) |
| *z* | - | Distance inside the porous of the solid matrix (m) |
| µg | - | microgram |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Research background

*Averrhoa bilimbi* Linn (Oxalidaceae) is a small–sized tree growing up to 15 m tall and 30 cm diameter. The chemical constituents of *A.bilimbi* that have been identified include amino acids, citric acid, cyanidin–3–O–β–D–glucoside, phenolics, potassium ion, sugars and vitamin A (Tan et al., 2005). It is used as antibacterial, antiscorbutic, astringent; post–partum protective medicine; treatment of fever, mumps, pimples, inflammation of the rectum and diabetes (decoction of the leaves); treatment of itches, boils, rheumatism, cough and syphilis (paste of leaves); treatment of scurvy, bilious colic, whooping cough, hypertension and as a cooling drink (juice of preserved fruits); treatment of children's cough (syrup of flowers); treatment of stomach ache (fruits) (Tan et al., 2005). Besides that, *A. bilimbi*, has been widely used in traditional medicine for cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough and hypertension (Abas et al., 2006).

**Figure 1.0** *Averrhoa Bilimbi* fruit (Sources: Sugeesh, 2008)

An increase in the consumption of fruits and vegetables is related with a decrease in the rate of cardiovascular disease and reduce risks of certain cancers. Fruits and derived products have a beneficial effect on the human health. Thus, *A. bilimbi* fruits have received much attention because of its nutritional and antioxidant properties. This is due mainly to the contribution of antioxidant compounds including vitamin C, phenolic compounds and carotenoids (Cano et al., 2008). The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, climatic conditions and cultural practices. Flavonoids contents in fruits mainly rely on genetic characteristics. Some analytical methods are applied to qualitative and quantitative flavonoid determination, especially by HPLC in conjunction with diode array detection and mass spectrometry for their identification and characterization.

Bioactive compounds evaluate from *Averrhoa bilimbi* are flavonoids, antioxidant capacity and phenolic. Flavonoids constitute the most common group of secondary plant metabolites that play important roles in the interactions of plants with their environments. The type, amount and localisation of flavonoids vary according to plant species and the developmental stage of the tissues, and may be modulated by environmental signals (Jeng et al., 2010). Ascorbic acid content is a powerful anti-

oxidant present in food and beverages, and it is also used as a marker chemical in evaluating food deterioration and product quality. Among the various bioactive substances, phenolic compounds which are plant secondary metabolites and have been proven to exhibit many health protective effects, have received most attention.

## 1.2 Problem statement

Results on quantification of bioactive compounds in *averrhoa bilimbi* will be varies due to different parameters on extraction. Optimum parameters on extraction are necessary to obtain the highest value in quantification of bioactive compounds which are total phenolics, total flavonoids and antioxidants capacity. Parameters that have been study in extraction includes effects on type of solvents with different concentration, effects of times, effects of temperatures and lastly effects of agitations. Basically, type of solvents with different concentration effects on extraction yield where the more polar the organic solvent, the more it is miscible or soluble in water hence resulting in a good extraction. Many authors established that the extraction yield of bioactive compounds is greatly depending on the solvent polarity (Turkmen et al., 2006; Lapornik et al., 2005). The difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and its extraction yield. While for the concentration solvents, (Rødtjer et al., 2006) reported that quantification of the total amount of phenolics in the extracts showed that 50% solvent-water mixtures extracted the phenolics more efficiently and contained more complex mixtures of phenolic compounds than the pure solvent extracts did. The difference in polarities of extracting

solvents might influence the solubility of chemical constituents in a sample and its extraction yield.

On top of that, bioactive compound would be higher if optimum time is used for the extraction. The time must be not too long or too short because it will absolutely effect on the amount of extraction. Prolongation of extraction duration potentially increases the loss of phenolics, flavonoids and antioxidants by exposure to light and oxygen. An increase in extraction time might give rise to possible degradation (Garcia-Sales et al., 2010). However, a short extraction time might yield a small amount of bioactive compounds. Thoo et al. (2010) revealed that excess extraction time lead to reduction of phenolic and antioxidant yields. Besides that, to achieve higher bioactive compound, optimum temperature is a crucial factor that is necessary to be observed. For higher temperature, the phenolic, flavonoids and antioxidants capacity will be degrades and become unstable and this resulting to the lower of extraction while for low temperature, the amount of extraction on the fruit cannot be done greatly and lead to small amount recorded. Lastly, effect of agitation on extraction is one of the important parameters to be focus. By considering all the optimum parameters, higher quantification of bioactive compounds can be achieved.

## 1.3 Research objective

The purpose of this study is to determine the bioactive compounds in *averrhoa bilimbi* and to study their optimum parameters during extraction.

## 1.4 Scope of research

The following are the scopes of this research to support the above mentioned objectives:

i) To analyze the exact amount and accurate data on how much the capacity of bioactive compounds which are flavonoids, phenolic, and antioxidant content in *A. bilimbi*.

ii) To study optimum parameters such as effects on type of solvents with different concentrations, effects of times, effects of temperatures and effects of agitations.

## 1.5 Rationale and significance

This study will provide a better understanding on the quantification of bioactive compounds in *A. bilimbi* by using different methods in testing each of bioactive compounds include determination of flavonoids, phenolic, and antioxidant activity with the optimum parameters on extraction where accurate result will be identify showing the right content of bioactive compound in *A. bilimbi* which is expected to be achieved upon the completion of this study.

## 1.6 Thesis outline

In this research, it will divide into five chapters. Firstly, Chapter 1 is an overview about this research. It consists of the introduction on *A. bilimbi* which gives a brief idea on what are bioactive compounds content in it. The problem statement, objective and the scope of the study also are included in this chapter.

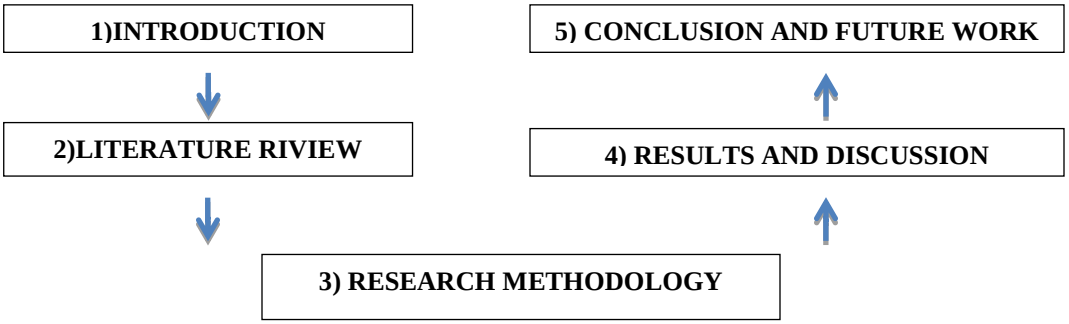


**Figure 1.2** The road map for thesis

Chapter 2 is about literature review on *A. bilimbi* fruit, list of method apply in quantifying bioactive compounds and what is the finding in each of the method. In this chapter, all the relevant technical paper, journals, and books taken from those researches will be studied and discussed.

Then, Chapter 3 will be covered the parts of experimental set up and will be explained more details on methodology and operating procedures. The techniques and the algorithms that will be used in performing this study will be applied. The method and techniques used for this system is described in detail. In addition, in this chapter also explained the material used in this experiment and the method use to analysis the data.

Chapter 4 will be covered on the results and discussion of the research during the operation process. All the experimental result and data will be discussed in details which are including the capacity each of the bioactive compounds contain in *A. bilimbi* based on the different parameters. The detailed report on the product quality analysis was evaluated. Implementation of process that is involved during development of this analysis is explained in detail in this chapter.

Chapter 5 will be discussed on the conclusion can be made for the study and some recommendations can be taken.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction and Historical Background

Food engineering is a multidisciplinary field of applied physical sciences which include science, microbiology, and engineering education for food and related industries. Food engineering includes, but is not restricted to, the application of agricultural engineering, mechanical engineering and chemical engineering principles to food materials. Food engineers offered the technological knowledge transfer essential to the cost-effective production and commercialization of food products and services. This type of technology is being used not only for production but in fact it can provide a good result in identifying and quantifying some compound in certain fruits, vegetables and others. The quantification process is determined by some methods present to determine certain bioactive compound in the fruit for example. We look into our material world through the methods and the tools of analytical chemistry. Those analytical techniques existing now are always being required to improve by analytical chemists to meet the

arising requirements for better chemical measurement from our society. Usually one or more standard specific procedures are accessible for determination of an analyte in a provided sample. However, it is not accurately can be accepted by the other analyte. The analyst needs to depend on his experience and knowledge to carry out and analytical method for a sample. Since in *A. bilimbi*, there are some kinds of bioactive compounds, some methods with a right reagent should be apply to determine each of bioactive compounds contain in it.

## 2.2 Averrhoa bilimbi

The bilimbi, *Averrhoa bilimbi,* L., (Oxalidaceae), is intimately allied to the carambola but quite dissimilar in appearance, behaviour of fruiting, flavour and uses. The only strictly English names are "cucumber tree" and "tree sorrel", bestowed by the British in colonial times. "*Bilimbi*" is the common name in India and has become extensively used. In Malaysia, it is called *belimbing asam, belimbing buloh, b'ling,* or *billing-billing* while in Indonesia, it is known as *belimbing besu, balimbing, blimbing,* or *blimbing wuluh;* in Thailand, it is famous with *talingpling,* or *kalingpring.*In Haiti, it is called *blimblin;* in Jamaica, *bimbling plum;* in Cuba, it is *grosella china;* in El Salvador and Nicaragua, *mimbro;* in Costa Rica, *mimbro* or *tiriguro;* in Venezuela, *vinagrillo;* in Surinam and Guyana, *birambi;* in Argentina, *pepino de Indias.* To the French it is *carambolier bilimbi,* or *cornichon des Indes.* Filipinos normally call it *kamias* but there are about a dozen other native names (Morton, 1987).

**Figure 2.1** Averrhoa Bilimbi fruit (Sources: Sugeesh, 2008)

### 2.2.1 Description of Averrhoa Bilimbi

Description of this fruit such as the tree is attractive, long-lived, reaches 16 to 33 ft (5-10 m) in height; has a short trunk soon separating into a number of upright branches. The leaves, very alike to those of the Otaheite gooseberry and mainly clustered at the branch tips, are alternate, imparipirmate; 12 to 24 in (30-60 cm) long, with 11 to 37 alternate or sub opposite leaflets, ovate or oblong, with rounded base and pointed tip; downy; medium-green on the upper surface, pale on the underside; 3/4 to 4 in (2-10 cm) long, 1/2 to 1 1/8 in (1.2-1.25 cm) wide. Small, fragrant, 5-petalled flowers, yellowish-green or purplish marked with dark-purple, are borne in small, hairy panicles emerging directly from the trunk and oldest, thickest branches and some twigs, as do the clusters of curious fruits. The *bilimbi* is ellipsoid, obovoid or just about cylindrical, faintly 5-sided, 1 1/2 to 4 in (4-10 cm) long; capped by a thin, star-shaped calyx at the stem-end and tipped with 5 hair-like floral remnants at the apex. The fruit is crisp when unripe, turns from bright-green to yellowish-green, ivory or nearly white when ripe and

falls to the ground. The outer skin is glossy, very thin, soft and tender, and the flesh green, jelly-like, juicy and extremely acid. There may be a few (perhaps 6 or 7) flattened, disc-like seeds about 1/4 in (6 mm) wide, smooth and brown.

### 2.2.2 Origin and Distribution

Perhaps a native of the Moluccas, the *bilimbi* is grown throughout Indonesia; is cultivated and semi-wild everywhere in the Philippines; is much grown in Ceylon and Burma. It is very familiar in Thailand, Malaysia and Singapore, common in gardens across the plains of India, and has run wild in all the warmest areas of that country. It is much planted in Zanzibar. Introduced into Queensland about 1896, it was readily adopted and commercially spread to growers. In 1793, the *bilimbi* was passed from the island of Timor to Jamaica and, after some years, *bilimbi* was planted in Cuba and Puerto Rico, Trinidad, the lowlands of Central America, Venezuela, Colombia, Ecuador, Surinam, Guyana and Brazil, and even in northern Argentina, and it is very famous among the Asiatic residents of those countries as it must be in Hawaii. Still it is grown only as an occasional interest in southern Florida.

### 2.2.3 Uses and applications

The *bilimbi* is usually regarded as too acid for eating raw, but in Costa Rica, the green, uncooked fruits are set up as a relish which is served with rice and beans.

Sometimes it is an accessory for fish and meat. Ripe fruits are often added to curries in the Far East. They yield 44.2% juice having a pH of 4.47, and the juice is famous for making cooling beverages on the order of lemonade. Mostly, the *bilimbi* is used in place of mango in making of chutney, and it is much preserved. To decrease acidity, it may be first pricked and soaked in water overnight, or soaked in salted water for a shorter period; then it is boiled with much sugar to make a jam or an acid jelly. The latter, in Malaysia, is putting to stewed fruits that are oversweet. Half-ripe fruits are salted, expose to the sun, and pickled in brine and can be thus kept for 3 months. A quicker pickle is made by place the fruits and salt into boiling water. This product can be kept only 4 to 5 days. The flowers are occasionally preserved with sugar.

For medical uses and application, the leaves are function as a paste or poultice on itches, swellings of mumps and rheumatism, and on skin eruptions in the Philippines. In another place, they are applied on bites of poisonous creatures. Malayans make used the leaves fresh or fermented as a treatment for venereal disease. A leaf infusion is a therapy for coughs and is taken after childbirth as a tonic. A leaf decoction is used to relieve rectal inflammation. A flower infusion is said to be helpful against coughs and thrush. In Java, the fruits combined with pepper are eaten to provide sweating when people are feeling "under the weather". A paste of pickled *bilimbis* is smeared all over the body to hurry the recovery after a fever. The fruit conserve is used as a treatment for coughs, beriberi and biliousness. Syrup prepared from the fruit is taken as a therapy for fever and inflammation and to stop rectal bleeding and alleviate internal haemorrhoids. It is used as antibacterial, ant scorbutic, astringent; post–partum protective medicine; treatment of fever, mumps, pimples, inflammation of the rectum and diabetes (decoction of the leaves); treatment of itches, boils, rheumatism, cough and syphilis (paste of leaves);

treatment of scurvy, bilious colic, whooping cough, hypertension and as a cooling drink (juice of preserved fruits); treatment of children's cough (syrup of flowers); treatment of stomach ache (fruits) (Tan et al., 2005). Besides that, *A. bilimbi*, has been widely used in traditional medicine for cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough and hypertension (Abas et al., 2006).

## 2.3 Method in quantifying of bioactive compounds in *A. bilimbi*

Some methods through analytical technique are applied in order to analyse and determine bioactive compounds contain in *A. bilimbi*. Choosing a right reagent is compulsory to be added in procedure leading an accurate data for result. Each of the bioactive compounds has their own identification method, reagent, conditions and technologies used. Below are the method selected for quantification of bioactive compound in *averrhoa bilimbi.*

## 2.4 Antioxidant capacity

Antioxidants can be classified within two classes as synthetic and natural. Among the synthetic types, the most commonly used to preserve food are butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate (PG) and tertbutyl hydroquinone (TBHQ). For the same function, tocopherol and ascorbic acid can be broadly used as natural antioxidants. Several reports reveal that BHA and